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Transfer of proteins from gels to diazobenzyloxymethyl-paper and detection with antisera: A method for studying antibody specificity and antigen structure

(simian virus 40/T antigens/virion proteins/reversible gel crosslinks/*Staphylococcus aureus* protein A)

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ABSTRACT **We describe a rapid and very sensitive method for detecting proteins as antigens after their separation in polyacrylamide/agarose composite gels, with or without sodium dodecyl sulfate. The polyacrylamide matrix is crosslinked with a reagent that can be cleaved with periodate or alkali to facilitate transfer of the protein bands to diazobenzyloxymethyl-paper, where they are coupled covalently. Specific proteins are detected by autoradiography after sequential incubation with unfractionated, unlabeled specific antiserum and ^{125}I-labeled protein A from *Staphylococcus aureus*. Antibody and protein A can be removed with urea and 2-mercaptoethanol, and the same paper can be probed again with a different antiserum. An antiserum specific for the simian virus 40 virion proteins VP3 and VP2 has been prepared; it does not crossreact with VP1, as demonstrated by this method. An antiserum raised in rabbits against simian virus 40-transformed rabbit kidney cells is shown to be directed primarily against a periodate-sensitive moiety present in tumor (T) antigen from infected or transformed cells, whereas an antiserum raised in rabbits against large T antigen purified from lytically infected monkey kidney cells by electrophoresis in the presence of sodium dodecyl sulfate [Lane, D. P. & Robbins, A. K. (1978) *Virology* 87, 182–193] is directed primarily against determinants that are not sensitive to periodate.**

Specific proteins have been detected in crude extracts by immunoprecipitation with specific antisera, followed by gel electrophoresis in the presence of sodium dodecyl sulfate ($NaDodSO_4$). The proteins are usually tagged with a radioisotope to facilitate detection and to distinguish them from the unlabeled proteins of the antisera. Studies of the tumor (T) antigens coded for by SV40 (1, 2) or polyoma (3, 4) provide recent examples of the use of this method. If the antigen is a member of a complex aggregate of different polypeptides, the entire aggregate will be precipitated and detected. Also, proteolytic degradation of the antigens in a crude extract can sometimes be a problem during the long incubation times necessary for complete immunoprecipitation. To circumvent complications such as these, alternative approaches were developed in which unlabeled proteins were first separated by gel electrophoresis and then detected by incubating the gel with radioactive antibody (5–7) or by transferring the bands of protein from the gel into an overlay of agarose impregnated with unlabeled serum (8). However, because careful washing is required to obtain good ratios of signal to background, these procedures are slow.

We now describe a modification of the approach of separating the unlabeled proteins first. The procedure is simple, rapid, and very sensitive, and it gives excellent ratios of signal to background. By using this procedure, we show that SV40 T antigen has periodate-sensitive groups which can be major antigenic determinants and that an antiserum directed against the purified SV40 virion protein VP3 does not crossreact with VP1.

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MATERIALS AND METHODS

Cells and Viruses. The CV-1 line of African monkey kidney cells and SV40 strain VA 45-54 (9) were used. SV40 virions were purified according to Christiansen *et al.* (10). TRK-54, an SV40-transformed line of rabbit kidney cells (11), was kindly provided by P. H. Black and H65-90B, an SV40-transformed hamster cell line (12), by S. Tevethia.

Preparative Separation of SV40 Virion Proteins. SV40 virus in 10 mM NaCl/10 mM Tris·HCl, pH 7.2/1 mM $MgCl_2$ was mixed with 0.1 vol of 1 M sodium phosphate, pH 6.85/7.5% $NaDodSO_4$/10 mM dithiothreitol and the virions were disrupted by heating the mixture at 80°C for 5 min. Solid urea was added to the clear solution to give a final concentration of 6 M and the sample was passed through a column of Dowex AG1-X8, bed volume 4 times the sample volume, previously equilibrated with buffer (0.13 M sodium phosphate, pH 6.85/8 M urea/10 mM 2-mercaptoethanol). The column flow-through was free of $NaDodSO_4$ and contained 90% of the input protein. It was loaded directly onto a column of Bio-Rex 70 equilibrated with the same phosphate/urea buffer, bed volume 2 times the sample volume. The column was then washed with about 15 column vols of this buffer until no further protein appeared in the flow-through. It was eluted first with buffer containing 1 M guanidinium chloride instead of urea and then with buffer containing 6 M guanidinium chloride. Separate pools of the flow-through plus wash fractions and the two guanidinium chloride eluates were desalted and concentrated in an Amicon ultrafiltration cell over a PM 10 membrane. The column buffer was exchanged for $NaDodSO_4$ gel sample buffer (13). Between 60 and 90% of the protein was recovered after this step. The concentrated flow-through plus wash fraction contains VP1 free of other viral proteins (Fig. 1, gel B), and the 1 M guanidinium chloride fraction contains residual VP1 and small amounts of VP3 plus some of the histones (Fig. 1, gel C). The bulk of the VP3 and the remaining histones are in the 6 M guanidinium chloride fraction, which is virtually free of contaminating VP1 (Fig. 1, gel D).

Rabbit Antisera. "SN" serum: A high titer anti-T serum was produced by injecting 1–2 × 10^7 TRK-54 cells (11) in Tris/saline buffer intramuscularly and subcutaneously into the backs of New Zealand White rabbits once a week for 3 weeks. Booster

Abbreviations: DATD, *N,N'*-diallyltartardiamide; DBM, diazobenzyloxymethyl; EDA, ethylene diacrylate; $NaDodSO_4$, sodium dodecyl sulfate; SV40, simian virus 40; TEMED, *N,N,N',N'*-tetramethylethylenediamine; T antigen, tumor antigen.

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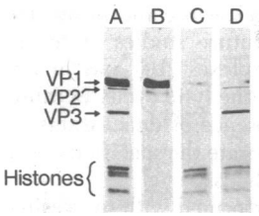

FIG. 1. Preparative separation of denatured SV40 virion proteins by chromatography on Bio-Rex 70. Aliquots of the concentrated column fractions were analyzed in a 15% polyacrylamide gel containing 0.8% bisacrylamide but no agarose. Electrophoresis was for 5 hr at 120 V and the gel was stained with Coomassie blue. Gels: A, SV40 virion proteins; B, pooled Bio-Rex 70 flow-through plus wash fractions; C, 1 M guanidinium chloride fraction; D, 6 M guanidinium chloride fraction.

injections were given 4 weeks later and the rabbits were bled after 1 week more. This procedure was repeated several times with each rabbit.

"LR" serum: Serum directed against large T antigen purified by gel electrophoresis in the presence of $NaDodSO_4$ (14) was the kind gift of D. P. Lane (Imperial Cancer Research Fund, London). This serum has been reported to react with both large T and small T antigens.

Anti-VP1 and anti-VP3 sera: The SV40 virion proteins, separated as described above, were purified further by gel electrophoresis in the presence of $NaDodSO_4$. Individual gel bands containing between 100 and 200 μg of protein were homogenized and injected into rabbits essentially as described by Tjian *et al.* (15).

Anti-SV40 virion serum: Purified virions (5 mg) in 1 ml of 25 mM Tris·HCl, pH 7.2/0.15 M NaCl/1 mM $MgCl_2$ were heated with 0.5% $NaDodSO_4$ for 5 min at 90°C. Freund's complete adjuvant (3 ml) and 2 ml of water were added and the emulsion was homogenized by sonication. A total of 2 ml was injected intramuscularly in several places along the back. Injections and bleedings were done on the same schedule used for preparing the "SN" anti-T serum.

All the antisera were titrated by using the protein A binding assay (16).

^{125}I-Labeled Proteins. Protein A from *Staphylococcus aureus* (Pharmacia) and proteins for use as molecular weight markers were iodinated by a modification of the procedure of Syvanen *et al.* (17) to specific activities of 5–15 μCi/μg (1 Ci = 3.7×10^{10} becquerels).

Gel Electrophoresis. Composite polyacrylamide/agarose slab gels were used with the discontinuous buffer system of Laemmli (13). Concentrated (30%) acrylamide stock solutions contained either 28.5 g of acrylamide and 1.5 g of *N,N'*-diallyltartardiamide (DATD) (Bio-Rad) (18) per 100 ml, or 28.9 g of acrylamide and 1.1 g of ethylene diacrylate (EDA) (Pfaltz & Bauer, Stamford, CT) (19) per 100 ml. To prepare the resolving gel, 6 ml of buffer containing 1.88 M Tris·HCl (pH 8.8), 11.5 ml of water, and 0.3 g of agarose were mixed and the agarose was dissolved by boiling. When the solution had cooled to 50°C, 12 ml of acrylamide stock solution, 0.3 ml of 10% $NaDodSO_4$, and the catalysts were added [DATD gels: 300 μl of 10% ammonium persulfate and 30 μl of *N,N,N',N'*-tetramethylethylenediamine (TEMED) per 30 ml of gel; EDA gels: 100 μl of persulfate and 10 μl of TEMED per 30 ml of gel]. The stacking gel contained no agarose and was always crosslinked with DATD, not EDA (see ref. 19). To prepare the stacking gel, 1 ml of acrylamide stock, 0.1 ml 10% $NaDodSO_4$, 1.25 ml of buffer containing 1 M Tris·HCl (pH 6.8), and 7.5 ml of water were mixed. The gel was polymerized by adding 100 μl of 10% ammonium persulfate and 10 μl of TEMED. Electrophoresis was carried out at room temperature at 120–180 V until the dye front reached the bottom of the gel.

Preparation of the Gel and Transfer. Diazobenzyloxymethyl (DBM)-paper was prepared according to Alwine *et al.* (20, 21). Diazotization of the paper at 0°C for 30–40 min should be timed so that the washed paper can be placed onto the prepared gel immediately. DATD gels were prepared for transfer by incubating them twice for 30 min each with 250 ml of 2% periodic acid (18), once for 15–30 min with 0.5 M sodium phosphate, pH 7.5, and twice for 15 min each with 50 mM sodium phosphate, pH 7.5, all with gentle rocking. EDA gels were prepared by incubating them twice for 30 min each with 0.25 M ammonium hydroxide, followed by the washes with sodium phosphate buffers described above. After the last wash, freshly prepared DBM-paper was placed onto the gel and the proteins were transferred by blotting overnight at room temperature, using 50 mM sodium phosphate, pH 7.5, essentially as described for DNA transfers by Southern (22). After transfer, the paper was incubated at 37°C for 2 hr with gentle rocking in 250 ml of 0.1 M Tris·HCl, pH 9.0/10% (vol/vol) ethanolamine/0.25% (wt/vol) gelatin to inactivate any remaining diazonium groups.

Identification of Specific Proteins Bound to DBM-Paper. The paper was incubated with antisera diluted 1:50 to 1:200 in buffer (150 mM NaCl/5 mM EDTA/50 mM Tris·HCl, pH 7.4) plus 0.25% gelatin and 0.05% Nonidet P-40 (23), 50–100 μl/cm^2, in plastic boiling bags (Seal-N-Save, Sears) with gentle rocking for 2–12 hr at 37°C. The paper was then washed for 2–12 hr at 37°C with 250 ml of the same buffer (without antiserum), incubated with ^{125}I-labeled protein A (0.25 μCi/gel slot) in the same way for 1–2 hr at 37°C, rinsed briefly with water, and washed for 2 hr in the same buffer containing 1 M NaCl and 0.4% (wt/vol) Sarkosyl instead of Nonidet P-40. The paper was rinsed with water, blotted dry, and autoradiographed, by using Kodak XR-5 film and a Du Pont Cronex Lightning-plus XL screen (24) at −70°C. The timing of the incubation and washing steps is very flexible; once the protein has been transferred, the paper can be stored at 4°C for at least 2 days after any of the steps above without affecting the results. The antibody and protein A can be removed by incubating the paper in a buffer containing 50 mM sodium phosphate (pH 7.5), 10 M urea, and 0.1 M 2-mercaptoethanol for 30 min at 60°C. After rinsing with water and then Tris buffer plus gelatin and Nonidet P-40, the paper can be incubated with a second antiserum and processed as described above.

RESULTS AND DISCUSSION

Transfer of Proteins to DBM-Paper. We have shown recently (21, 25) that small fragments of DNA can be separated on composite polyacrylamide/agarose gels containing DATD crosslinks and that the DNA fragments can be transferred to DBM-paper after cleavage of the crosslinks with periodate. The same approach can be used to transfer proteins from composite gels to DBM-paper. The efficiency of transfer of several proteins (M_r range 18,000–94,000) from a composite gel containing 12% acrylamide varied between 11 and 16% (Table 1). It is clear that a substantial fraction of each protein remains in the gel and that not all the protein that leaves the gel becomes covalently attached to the paper. Although there is room for improving the efficiency of transfer, the method is now sensitive enough that 100 pg of antigen or less can be detected (see below).

Experiments with SV40 Virion Proteins. As shown in Fig. 2, lane A, an antiserum directed against $NaDodSO_4$-denatured SV40 virions reacts with the three capsid proteins VP1, VP2, and VP3 and with smaller amounts of higher molecular weight species, as observed by Kasamatsu and Flory (6). The host histones present in the virions do not react. Anti-VP1 serum reacts

Table 1. Efficiency of transfer of different proteins to DBM-paper

Protein	Molecular weight	Percentage left in the gel	Percentage transferred to paper
Phosphorylase a	94,000	60	11
Bovine serum albumin	68,000	54	14
Glutamate dehydrogenase	53,000	55	14
Creatine kinase	40,000	42	15
Myoglobin	18,000	21	16

One microliter of a mixture of ^{125}I-labeled proteins (6.2 μCi/μg, 7 μg of each protein per ml) in 50 μl of gel sample buffer was run in each of two slots of a 12% acrylamide/agarose composite gel crosslinked with DATD. One track was dried and the other was transferred. After autoradiography, individual bands were cut from the dried gels (without transfer and after transfer) and from the paper, and the amount of radioactivity in each band was determined.

both with VP1 and with the higher molecular weight species, but not with VP3 (Fig. 2, lane B). Anti-VP3 serum reacts both with VP3 and VP2, but not with VP1 or with the higher molecular weight species (Fig. 2, lane C). As shown in Fig. 2, lane D, T antigen was not detected in the virions. However, a single molecule of T antigen per virion would have been below the limit of detection. Some of the larger species detected with anti-virion and anti-VP1 sera might be dimers and higher oligomers of VP1. Reaction of the anti-VP3 serum with VP2 is expected because the amino acid sequence of VP3 is contained entirely within the larger VP2 polypeptide (ref. 26 and references therein).

We describe here the preparation of an antiserum specific for VP2/VP3. Consigli and coworkers have separated the denatured proteins of polyoma virions according to size on Na-DodSO$_4$ gels (27) or on Sepharose columns in the presence of 6 M guanidinium chloride (28) and have raised antibodies against the separated gel bands. However, they have not shown that the antibodies are specific by evaluating their reactivities towards heterologous gel bands. Previous attempts using SV40 VP3 separated only by gel electrophoresis in the presence of NaDodSO$_4$ were unsuccessful due to crosscontamination by peptides related to VP1, which is an excellent antigen (T. Landers and P. Berg, personal communication). Prior purification by column chromatography in the presence of denaturants, followed by gel electrophoresis, gives VP3 of sufficient purity to generate a specific antiserum. This serum should be very valuable in future studies of the location of VP3 in nucleoprotein complexes (10) and in investigations of the function of VP3 in the lytic cycle. Because VP1 and VP3 are insoluble in the absence of denaturants, it would have been difficult to demonstrate the specificity of the anti-VP3 serum by alternative methods.

Experiments performed with increasing dilutions of SV40 virus indicate that VP1 can be detected with antivirion serum when as little as 100 pg of virions are loaded onto a single slot of the gel. Of course, the sensitivity will vary with the titer of the serum, the specific radioactivity of the protein A, and the time of exposure.

Detection of T Antigen in NaDodSO$_4$ Gels. Reaction of anti-T serum SN with transfers from EDA gels of total extracts from lytically infected and uninfected monkey cells are shown in Fig. 3. For the infected cells, serum SN reacts mainly with polypeptides of M_r 85,000 and 20,000 and less with polypeptides of M_r 68,000 and 55,000 (Fig. 3, lane A). The species of MW 68,000 is also detected with serum SN in extracts of uninfected CV-1 cells (Fig. 3, lane B), suggesting that it might be a cellular protein that crossreacts with anti-T serum. These experiments illustrate the specificity of the method, which gives a clear result even in the presence of all the cellular proteins. The 20,000-M_r polypeptide is likely to be small T antigen, because it is not found in uninfected cells. Preliminary results also suggest that it is absent in SV40 deletion mutants that map in the proximal part of the early region. Small T antigen has been shown by others (2) to have an M_r = 17,000; the difference in molecular weight is probably due to the use of different conditions for electrophoresis.

The sensitivity of the transfer technique was compared with that of a standard immunoprecipitation method by using extracts of cells labeled with [^{35}S]methionine (30). Extracts were immunoprecipitated with anti-T serum SN (Fig. 4, lane A) or normal rabbit serum (Fig. 4, lane B), using fixed *S. aureus* cells (23). Radioactive bands were detected by fluorography (31) after electrophoresis. In parallel, another portion of the same extract was fractionated by electrophoresis in a polyacrylamide/agarose gel containing EDA, transferred to DBM-paper, and detected with anti-T serum SN and ^{125}I-labeled protein A (Fig. 4, lane C). A portion of an extract from uninfected CV-1 cells was run and transferred in parallel (Fig. 4, lane D). The gel transfer technique yielded a clear signal for a protein of M_r about 85,000 (probably large T antigen) using an exposure time

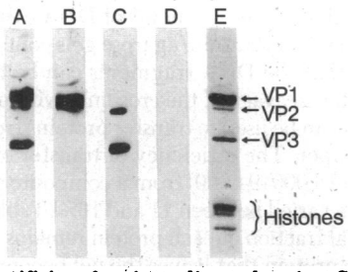

FIG. 2. Specificity of antisera directed against SV40 virion proteins. Purified SV40 virions (50 μg) were denatured by boiling in NaDodSO$_4$ gel sample buffer and the proteins were separated in a 15% polyacrylamide/DATD gel containing 1% agarose. Electrophoresis was at 120–150 V for 5 hr. One track was stained with Coomassie blue and the others were transferred to DBM-paper. The paper was cut and individual strips were exposed to rabbit antiserum (1:50 dilution) and labeled protein A. Exposure was for 30 min at −70°C. Lanes: A, antiserum against NaDodSO$_4$-disrupted SV40 virus; B, anti-VP1 serum; C, anti-VP3 serum; D, rabbit anti-T serum SN; E, stained gel track.

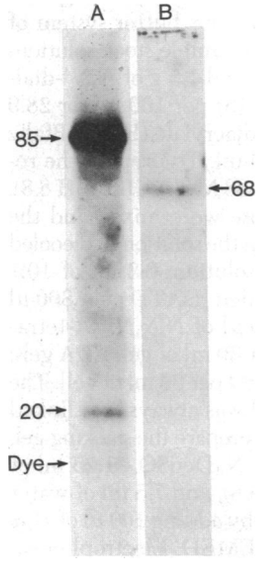

FIG. 3. Characterization of T antigens from SV40-infected cells. CV-1 cells were infected for 45 hr with 100 plaque-forming units of virus per cell. Extracts were prepared (29), and portions corresponding to 5 × 10^5 cells were fractionated by electrophoresis through a 12% polyacrylamide/agarose gel containing EDA for 5 hr at 120–150 V. The proteins were transferred to DBM-paper and the T antigens were identified by using anti-T serum SN (1:50 dilution). Autoradiography was carried out for 2 hr at −70°C. Lanes: A, infected cells; B, uninfected cells. The numbers refer to the molecular weights (×10^{-3}) of the proteins.

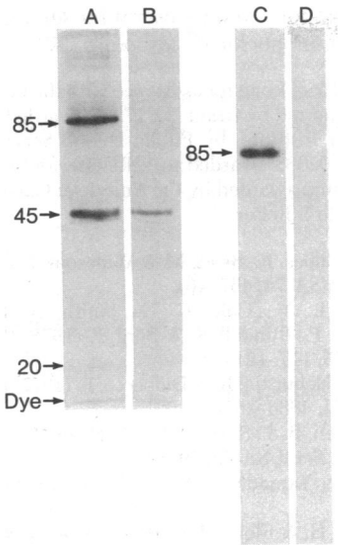

FIG. 4. Comparison of immunoprecipitation of [^{35}S]methionine-labeled extracts and gel transfers. CV-1 cells were infected for 48 hr with 100 plaque-forming units of virus per cell and labeled with 250 μCi of [^{35}S]methionine per 10^7 cells in Tris-buffered saline for 60 min at 37°C. The label was removed, medium containing 5% fetal calf serum was added, and the cells were incubated for 60 min more at 37°C. The medium was removed and the label was added back to the cells, which were incubated for another 60 min at 37°C. Cell extracts were prepared (30), and portions corresponding to 10^6 cells were immunoprecipitated with 25 μl of anti-T serum SN or normal rabbit serum as described by Collett and Erikson (29). The immunoprecipitated samples were analyzed by electrophoresis through a 12% polyacrylamide gel containing 0.8% bisacrylamide but no agarose at 130–150 V for 5 hr. The gel was stained with Coomassie blue, destained, and fluorographed (31). In parallel, portions of the same extract corresponding to 5×10^5 cells were fractionated by electrophoresis through a 12% polyacrylamide/agarose composite gel containing EDA for 4.5 hr at 130 V. The proteins were transferred to DBM-paper and the T antigens were identified with anti-T serum SN (1:50 dilution). (Lane A) [^{35}S]Methionine-labeled infected CV-1 cells, anti-T serum. The gel was fluorographed and exposed for 20 hr at −70°C. (Lane B) [^{35}S]Methionine-labeled infected CV-1 cells, normal rabbit serum; fluorography and exposure as in A. (Lane C) Infected CV-1 cells, transfer to DBM-paper, probed with anti-T serum, exposed for 20 min at −70°C. (Lane D) Uninfected CV-1 cells, transfer to DBM-paper, probed and exposed as in C. The numbers refer to the molecular weights ($\times 10^{-3}$) of the proteins.

of 20 min, whereas with immunoprecipitation and fluorography exposure times of 20 hr or more were required to produce a signal of comparable intensity.

Detection of Denatured and Native T Antigens from SV40-Transformed Hamster Cells. The reaction with anti-T serum SN of T antigen purified partially from nuclei of H65-90B cells by ammonium sulfate precipitation and chromatography on DEAE-cellulose (32) is shown in Fig. 5, lane A. The portion of the T antigen peak used was eluted at 0.2 M NaCl and was enriched for the 20,000-M_r species. The main peak of T antigen was eluted at 0.15 M NaCl and contained considerably less of the 20,000-M_r polypeptide. In addition to the 20,000-M_r species, a protein with M_r 85,000 (probably large T antigen) and a small amount of material of M_r 68,000 are also evident. The possibility that the 20,000-M_r species may be small T antigen is currently being investigated.

The immunodetection method can also be used with proteins separated in gels without denaturation. Partially purified T antigen was separated in agarose gels under the low salt conditions used by Varshavsky *et al.* (33) for the separation of SV40 nucleoprotein complexes. As shown in Fig. 5, lane B, T antigen from H65-90B cells migrates as at least three distinct species under these conditions. This result is in agreement with findings

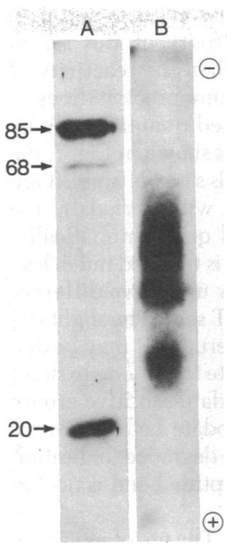

FIG. 5. Detection of denatured and native T antigens from SV40-transformed hamster cells. (Lane A) With $NaDodSO_4$. Partially purified T antigen from H65-90B cells was separated in a $NaDodSO_4$/12% polyacrylamide/agarose composite gel containing EDA and transferred to DBM-paper. After transfer, the paper was incubated with anti-T serum SN (1:50 dilution) and labeled protein A. The film was exposed for 5 hr at −70°C. (Lane B) Native. The same preparation of T antigen was separated by electrophoresis in a 1% agarose gel according to Varshavsky *et al.* (33). After electrophoresis for 2 hr at 1.25 mA the gel was incubated in 50 mM sodium phosphate, pH 7.5, for 1 hr and transferred to DBM-paper. The paper was incubated with serum SN (1:50 dilution) and labeled protein A. After being washed, the paper was exposed overnight at −70°C. The direction of migration was from top to bottom. The numbers refer to the molecular weights ($\times 10^{-3}$) of the proteins.

of Potter *et al.* (34), who showed that T antigen sediments as several different species of high molecular weight in sucrose gradients.

Different Anti-T Sera Are Directed against Different Antigenic Determinants. When T antigens were separated in gels crosslinked with EDA, the intensity of the signal for large T was similar with antisera SN or LR (Fig. 6, lanes A and B). However, when DATD gels were used, the intensity of the signal with serum LR was virtually unaffected (Fig. 6, lane D), but the intensity with serum SN was reduced dramatically (lane C). The reactivity of serum SN towards small T antigen was also abolished when extracts were analyzed by using DATD gels, as shown in Fig. 6, gel C. These results suggest that serum SN may recognize a periodate-sensitive antigenic determinant of T antigen, because periodate was used to cleave the DATD

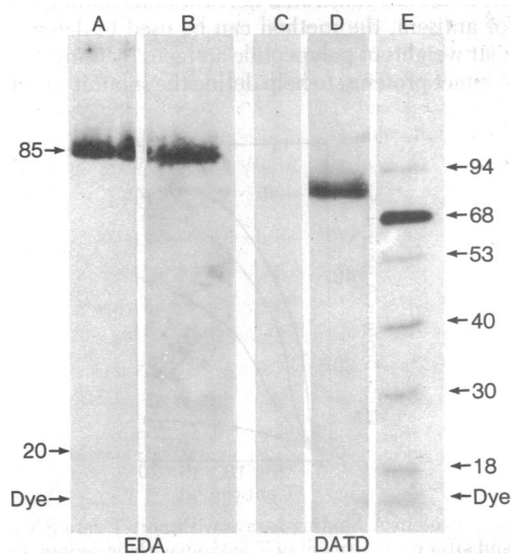

FIG. 6. Detection of SV40 T antigens after transfer from acrylamide gels containing DATD or EDA. SV40-infected CV-1 cells were extracted as described by Collett and Erikson (29). Portions corresponding to 6×10^5 infected cells were fractionated by electrophoresis on 12% polyacrylamide/agarose composite gels containing DATD or EDA for 5 hr at 120–150 V. The proteins were transferred to DBM-paper, and T antigens were identified with the two anti-T sera (1:120 dilution) and labeled protein A. Exposures were for 3 hr at −70°C. Lanes: A, EDA gel and serum SN; B, EDA gel and serum LR; C, DATD gel and serum SN; D, DATD gel and serum LR; E, molecular weight markers. The numbers refer to the molecular weights ($\times 10^{-3}$) of the proteins.

crosslinks in the gels. Small T antigen was not detected with serum LR, probably because the reactivity of this serum towards small T is considerably lower than the reactivity of serum SN, as judged from standard immunoprecipitations.

The effect of periodate was determined quantitatively by using the protein A binding assay (16). As shown in Fig. 7, the reactivities of untreated T antigen towards sera SN and LR are very similar. However, after treatment with periodate, the reactivity towards serum SN is reduced quite dramatically, whereas the reactivity towards serum LR is reduced much less. Results similar to these were obtained by using two different rabbit anti-T sera and two hamster anti-T sera. The slight decrease in reactivity of T antigen toward serum LR may be due to some loss of antigen during the periodate treatment, to slight reactivity of serum LR toward the periodate-sensitive group, or both. T antigen pretreated with periodate before electrophoresis in EDA gels does not appear to be degraded, indicating that cleavage of a periodate-sensitive peptide bond is not responsible for the loss of antigenicity.

Further Comments on the Technique. The use of iodinated protein A as a probe for the Fc portions of free IgG and immune complexes (for a recent review see ref. 35) eliminates the need to purify a specific gamma globulin from an immune serum, because only the specific gamma globulin will bind to the insoluble antigen. Also, only a very small amount of immune serum is required. In an alternative technique for detection of specific gel bands, $F(ab)'_2$ fragments are first linked to DBM-paper, followed by transfer and incubations with unlabeled gamma globulin and labeled protein A (36).

In addition to the examples described here, we have used the method successfully to detect the G protein of vesicular stomatitis virions, proteins from *Escherichia coli* (*dnaB* gene product, DNA binding protein, and *rep* protein), proteins from *Myxococcus xanthus* (with F. J. Murillo), and the 200,000-M_r multifunctional polypeptide responsible for the first three steps in the synthesis of UMP in hamster cells (data not shown). In addition to the use illustrated here for determining the specificity of antisera, the method can be used to determine the molecular weights of polypeptide antigens by using sera raised against intact proteins, to help define the subunit structures of such proteins. It may also be useful for screening hybrid cell lines producing monoclonal antibodies (37).

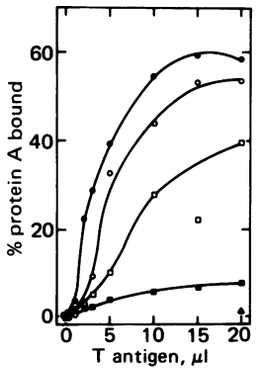

FIG. 7. Protein A binding assays with anti-T sera SN and LR before and after pretreatment of T antigen with periodate. Partially purified T antigen from H65-90B cells in a buffer containing 15 mM NaCl, 0.5 mM EDTA, and 5 mM Tris·HCl (pH 7.4) was treated with 15 mM $NaIO_4$ for 4 hr at 4°C. Another sample was incubated identically but without $NaIO_4$. Various amounts of treated and untreated T antigens were reacted with constant amounts of serum SN or LR at room temperature for 24 hr, as described by Crawford and Lane (16). The immune complexes were trapped on GF/C glass fiber filters and exposed to labeled protein A. The filters were washed and the amount of labeled protein A bound was measured. ●, Untreated sample and serum SN; ○, untreated sample and serum LR; ■, $NaIO_4$-treated sample and serum SN; □, $NaIO_4$-treated sample and serum LR; ▲, control without serum.

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